Research Report

Toward a comparison of microelectrodes for acute and chronic recordings

Matthew P. Ward\textsuperscript{a,*}, Pooja Rajdev\textsuperscript{a}, Casey Ellison\textsuperscript{a}, Pedro P. Irazoqui\textsuperscript{a,b}

\textsuperscript{a}Weldon School of Biomedical Engineering, Purdue University, MJIS 2083, 206 S Martin Jischke Drive West Lafayette, IN, 47907 USA
\textsuperscript{b}School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN, USA

\textbf{ARTICLE INFO}

\textbf{ABSTRACT}

Several variations of microelectrode arrays are used to record and stimulate intracortical neuronal activity. Bypassing the immune response to maintain a stable recording interface remains a challenge. Companies and researchers are continuously altering the material compositions and geometries of the arrays in order to discover a combination that allows for a chronic and stable electrode–tissue interface. From this interface, they wish to obtain consistent quality recordings and a stable, low impedance pathway for charge injection over extended periods of time. Despite numerous efforts, no microelectrode array design has managed to evade the host immune response and remain fully functional. This study is an initial effort comparing several microelectrode arrays with fundamentally different configurations for use in an implantable epilepsy prosthesis. Specifically, NeuroNexus (Michigan) probes, Cyberkinetics (Utah) Silicon and Iridium Oxide arrays, ceramic-based thin-film microelectrode arrays (Drexel), and Tucker-Davis Technologies (TDT) microwire arrays are evaluated over a 31-day period in an animal model. Microelectrodes are compared in implanted rats through impedance, charge capacity, signal-to-noise ratio, recording stability, and elicited immune response. Results suggest significant variability within and between microelectrode types with no clear superior array. Some applications for the microelectrode arrays are suggested based on data collected throughout the longitudinal study. Additionally, specific limitations of assaying biological phenomena and comparing fundamentally different microelectrode arrays in a highly variable system are discussed with suggestions on how to improve the reliability of observed results and steps needed to develop a more standardized microelectrode design.

\textbf{Keywords:}
Microelectrode
Signal-to-noise ratio
Disease
Prosthesis
Inflammation
Epilepsy

\textbf{1. Introduction}

In recent years, several microelectrode array (MEA) configurations and insertion techniques have been developed and refined in hopes of developing a long-term, stable communication interface with the brain (Hoogerwerf and Wise, 1994; Jones et al., 1992; Maynard et al., 1997; Moxon et al., 2004b; Nicolelis et al., 2003; Normann et al., 1999; Rennaker et al., 2005; Rousche and Normann, 1992). Many of these designs are capable of establishing interfaces with neuronal populations of interest from which high signal-to-noise ratio (SNR) recordings can be obtained for several months to years (Maynard et al., 1997; Nicolelis et al., 2003; Rousche and Normann, 1998; Vetter et al., 2004). Much of the new technology, however, does not supersede traditional microwire technology in its ability to evade a host immune response.

\textsuperscript{*}Corresponding author. Fax: +1 765 494 1912.
E-mail addresses: mpward@purdue.edu, mpward@mac.com (M.P. Ward).

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response. Researchers hope to develop MEAs capable of consistently recording signals from single or small groups of neurons indefinitely without losing microstimulation capabilities, as this will bring them one step closer to developing neural prostheses that can function for the lifetime of an implantee (Nicolelis, 2001; Schwartz, 2004).

Significant contributions to MEA failure have been linked to the host’s response to traumatic brain injury (TBI) resulting from insertion and a long-term foreign body response to the implant (Holecko et al., 2005; Rennaker et al., 2005; Turner et al., 1999). The initial injury from insertion, caused by all invasive electrodes, activates a cascade of events that ultimately result in the formation of a loose sheath of microglia, astrocytes, various extracellular matrix constituents, and macrophages around the implant (Biran et al., 2005; Kim et al., 2004b; Kreutzberg, 1996; Rennaker et al., 2005; Turner et al., 1999). During the first weeks to months following implantation, the sheath becomes more compact and fibrous, effectively isolating the MEA from the neural tissue of interest (Liu et al., 1999). Roitbak and Sykova (1999) suggest that the glial scar compromises recording quality by impeding ion diffusion through the extracellular space. Concurrent with the glial scar formation, neuronal density within the recording radius of the microelectrodes is reduced, leading to even fewer distinguishable single-unit recordings (Biran et al., 1999; Biran et al., 2005; Edell et al., 1992; Kim et al., 2004a; Kim et al., 2004b; Polikov et al., 2005; Purcell et al., 2009; Turner et al., 1999). The links between these phenomena are not well characterized, however, impeding the progression of MEA technology (Purcell et al., 2009; Roitbak and Sykova, 1999).

Popular and relatively successful MEAs today have significantly different material compositions, geometries, manufacturing processes, degrees of invasiveness, and degrees of biocompatibility (Donoghue, 2002; Maynard et al., 1997). The majority of them are reported to fail within weeks to months after implantation (Kipke et al., 2003; Nicolelis et al., 2003; Rousche and Normann, 1998; Vetter et al., 2004). Some current research aims to characterize and optimize individual aspects of the MEA designs that are believed to contribute most significantly to their failure, namely substrate composition, geometry, surface features, and insertion techniques (Biran et al., 1999; Holecko et al., 2005; Moxon et al., 2004a; Rennaker et al., 2005; Seymour and Kipke, 2007; Turner et al., 1999). Other research aims to develop electrodes with alternative configurations and applied polymer and drug-eluting coatings (Ludwig et al., 2006; Moxon et al., 2004a; Purcell et al., 2009; Yang and Martin, 2004). To date, no MEA configuration has successfully evaded the foreign body response.

A detailed understanding of the specific features of MEAs that contribute to their failure is needed in order to move towards a more standardized microelectrode design for chronic applications (Biran et al., 1999; Purcell et al., 2009; Silver and Miller, 2004). The first step toward this goal is to find reliable methods to quantify the wound-healing response around a chronically implanted MEA. One promising method assays the electrical properties of the tissues surrounding the implant. Because the fibrous encapsulation is much less conductive than healthy neural tissue, impedance measurements can be used to track the extent and progress of the immune response (Johnson et al., 2005; Otto et al., 2006; Williams et al., 2007). The extent of encapsulation can later be confirmed with histological analyses of the tissues surrounding the implants (Kim et al., 2004b; Kreutzberg, 1996; Rennaker et al., 2005; Turner et al., 1999).

This study is an initial effort comparing several commercially available MEAs with varying compositions and geometries in a rat model (Fig. 1). Tables 1 and 2 list basic attributes of the arrays compared, including MEAs from Cyberkinetics Neurotechnology Systems, NeuroNexus Technologies, Drexel University, and Tucker-Davis Technologies. To accurately assess the scarring response from these MEAs in an animal model with a high level of confidence, variation within and between treatment groups (treatment=MEA type + recommended insertion protocol) should be well controlled using a standardized study design. Controlling for variation within an animal study such as the one proposed is extremely difficult, however, due to the large numbers of animals required in each experimental group, the high costs of MEAs, the markedly different MEA configurations and insertion protocols, and a highly limited experimental budget. Ideally, each MEA should be evaluated side-by-side in the same brain region using identical surgical procedures and several repetitions. Such a design helps minimize biological and procedural variability, but does not allow each MEA to perform at its optimal level when using the same implantation procedures, given that specific insertion procedures are in place to maximize functionality. Additionally, the small size of a rat brain and the fundamentally different MEA designs further preclude this option, necessitating the need for a large-scale study to draw significant conclusions.

A simple experimental design was chosen to keep the preliminary study costs to a minimum, highlighting the large amount of variability in such comparative in vivo central nervous system study designs and the need for well-controlled, large-scale studies to evaluate specific biological responses. Despite a high degree of variability, this preliminary study was able to capture critical properties of each MEA and limitations of using only impedance measurements over time as a correlate for evaluating the scarring response. It is hoped that this initial study will facilitate funding for a follow-up study so that larger sample sizes and more MEA types can be evaluated in a more controlled manner.

In addition to discussing the optimal application of each MEA, insights are provided into 1) major sources of variation in these types of study designs, 2) whether evaluating the foreign body response in vivo using only impedance analyses yields enough information to assess MEA performance, and 3) possible approaches for generating a standardized MEA design that remains functional indefinitely.

To determine baseline properties and overall site-to-site variability, MEA impedance and charge capacity are first evaluated in 1× phosphate-buffered saline (1× PBS). Up to 4 MEAs of a particular geometry are then assessed over a 31-day period in a rat model with routine analyses of site impedances and single-unit recording capabilities. After the 31-day implant period, a histological analysis of the explanted brain is performed. Section 2 provides an organized review of data collected throughout the study. Section 3 discusses the limitations of using only impedance as a correlate for the scarring response, steps needed to develop improved MEA
technology, sources of variability within the study design, strengths and/or weaknesses of each MEA investigated, key findings, and unexpected results. Section 4 provides an overview of the methods used to evaluate each MEA, including MEA-specific surgical procedures, data collection procedures, histology protocols, and data analysis schemes.

2. Results

2.1. Pre-characterization

In this study, charge capacity is defined as a measure of an electrode’s ability to deliver charge in a reversible manner to neural tissue. An electrode cannot deliver more charge than its measured capacity without succumbing to damage by oxidative or reductive mechanisms (Cogan, 2008; Troyk et al., 2007). To avoid further decreasing sample sizes, this study only reports the reversible charge injection limits and not the limits of voltage application before overpotential and galvanic corrosion occur (McNaughton and Horch, 1996). Wide distributions of charge capacities and impedances were observed from the cyclic voltammetry and frequency response analyses (CV and FRA, respectively). A summary of the 1× PBS pre-characterizations is presented in Table 3. Averaged CV plots are seen in Figs. 2B, D, F, H, and J.

The Cyberkinetics Iridium Oxide MEA exhibited the greatest charge capacity ($Q_{\text{cap}}$) with a mean of 10.4±0.84 mC/cm². The NeuroNexus MEA exhibited the smallest charge capacity with a mean of 0.8±0.02 mC/cm². Normalized to recording site surface area, the Cyberkinetics Silicon MEA had the largest 1 kHz baseline impedance with a mean of 25.5±1.47 GΩ/cm². The TDT Microwire MEA had the lowest 1 kHz impedance with a mean of 0.87±0.04 GΩ/cm².

The presented charge capacity values represent the maximum amount of charge per unit area that can be delivered in the cathodal phase of a biphasic stimulation waveform. The area of the cathodal phase of a biphasic current pulse normalized to electrode surface area should be below the reported value of charge capacity to avoid damaging the electrode or tissue of interest. However, due to variable impedances from the glial scar and an in vivo ionic environment that differs greatly from 1× PBS, the reported charge injection limits must be used carefully (Cogan, 2008).

2.2. Electrical analysis of reactive tissue response

Electrochemical impedance spectroscopy (EIS) was used to measure electrode impedances from 0.1 to 10 kHz on the day of implantation and on days 1, 2, 3, 5, 7, 10, 15, 20, 25, and 30 following implantation (refer to Table 4 for details on animals included in the analysis). The 1 kHz impedance trends were...
### Table 1 – Relevant microelectrode array specifications.

<table>
<thead>
<tr>
<th>MEA</th>
<th># Sites</th>
<th>Site layout</th>
<th>Site/probe geometry</th>
<th>Substrate material</th>
<th>Recording site material</th>
<th>Recording site dimensions</th>
<th>Pitch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyberkinetics a,b Neurotechnology Systems, Inc. Iridium Oxide Array</td>
<td>16</td>
<td>1 site per shank</td>
<td>Shank length: 1 mm Base: 1.6 mm Conical recording site</td>
<td>Boron doped</td>
<td>Silicon w/ parylene C insulation</td>
<td>Radius: 3–5 μm</td>
<td>400 μm</td>
</tr>
<tr>
<td>Cyberkinetics Neurotechnology Systems, Inc. Silicon Array (Utah array) (Campbell et al., 1991; Rousche and Normann, 1998)</td>
<td>32</td>
<td>1 site per shank</td>
<td>Shank length: 1 mm Base: 2.4 mm Conical recording site</td>
<td>Boron doped</td>
<td>Silicon w/ parylene C insulation</td>
<td>Length: 35–75 μm</td>
<td>Site area: ~760 μm²</td>
</tr>
<tr>
<td>Moxon Thin-Film Ceramic Array (Moxon et al., 2004a; Moxon et al., 2004b)</td>
<td>4</td>
<td>4 sites linearly arranged on a single shank</td>
<td>Shank length: 7 mm Probe thickness: 44 μm (Varies: 38–50 μm)</td>
<td>Ceramic Cr, Pt</td>
<td></td>
<td>Site area: ~1760 μm²</td>
<td>200 μm</td>
</tr>
<tr>
<td>NeuroNexus Silicon Array (Michigan microelectrode system) (Hetke et al., 1994; Kipke et al., 2003)</td>
<td>16</td>
<td>16 sites linearly arranged on 4 shanks</td>
<td>Shank length: 3 mm Probe thickness: 15 μm</td>
<td>Silicon w/ Silicon dioxide/ nitride insulation</td>
<td>Ir</td>
<td>Site area: ~1250 μm² (along shank)</td>
<td></td>
</tr>
<tr>
<td>Tucker-Davis Technologies, Inc. (TDT) Microwire Array (<a href="http://www.tdt.com">http://www.tdt.com</a>)</td>
<td>16</td>
<td>1 site per shank</td>
<td>Wire diameter: 50 μm Tip angle: 45°</td>
<td>Polyimide insulation</td>
<td>W</td>
<td>Site area: ~2300 μm²</td>
<td>250 μm</td>
</tr>
</tbody>
</table>

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### Other configurations exist for Cyberkinetics, NeuroNexus, and TDT arrays, but these were chosen as representatives of the whole. Recording site area refers to the geometric surface area for an individual electrode on an MEA, not the size of the brain region from which it can record. Pitch refers to the spacing between adjacent electrode sites on an MEA.

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### Table 2 – Microelectrode array applications.

<table>
<thead>
<tr>
<th>MEA</th>
<th>Manufacturer suggested applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyberkinetics IrOx</td>
<td>• Chronic recording and stimulation in a single layer 1 to 1.5 mm below the cortex surface • See Cyberkinetics Silicon below • MEA type: Floating • Insertion method: Fast (&gt;8.3 m/s to insert a 100 electrode array to a depth of 1.5 mm) using Cyberkinetics' Pneumatically-actuated Impulse Microelectrode Array Inserter (Rousche and Normann, 1992)</td>
</tr>
<tr>
<td>Cyberkinetics Silicon (Campbell et al., 1991; Rousche and Normann, 1998)</td>
<td>• Chronic recording and stimulation in a single layer 1 to 1.5 mm below the cortex surface • Various applications in sensory, visual, and motor cortex research • Various applications in spinal cord, association area, and peripheral nerve fiber research • Previously used in primates, felines, and rodents • MEA type: Floating • Insertion method: Fast (&gt;8.3 m/s to insert a 100 electrode array to a depth of 1.5 mm) using Cyberkinetics' Pneumatically-actuated Impulse Microelectrode Array Inserter (Rousche and Normann, 1992)</td>
</tr>
<tr>
<td>Moxon Thin-Film Ceramic a,b (Moxon et al., 2004a; Moxon et al., 2004b)</td>
<td>• Chronic unit recordings across multiple layers of cortex or hippocampus • Previously used in rodents • MEA type: Fixed • Insertion method: Slow (~10 μm/min) with micromanipulator</td>
</tr>
<tr>
<td>NeuroNexus Silicon (Hetke et al., 1994; Kipke et al., 2003)</td>
<td>• Chronic unit recordings from multiple layers of cerebral cortex with stimulation capabilities • Previously used in mammals, fish, and insects in both central and peripheral nervous system research • MEA type: Floating • Insertion method: Slow (hand insertion) using Teflon-coated forceps</td>
</tr>
<tr>
<td>TDT Microwire (<a href="http://www.tdt.com">http://www.tdt.com</a>)</td>
<td>• Chronic unit recordings from multiple layers of interest along with stimulation capabilities (with custom configurations) • MEA type: Fixed • Insertion method: Slow (50–100 μm increments) with micromanipulator (Kralik et al., 2001)</td>
</tr>
</tbody>
</table>

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*a Moxon Thin-Film Ceramic MEAs are not commercially distributed, but were included in this study due to their unique design and material composition and the generosity of the developers. These MEAs were developed at Drexel University by KA Moxon, SC Leiser, GA Gerhardt, KA Barbee, and JK Chapin.
followed through the duration of implantation to monitor the impedance, and by inference, the extent of electrode encapsulation (Figs. 2A, C, E, G, and I) (Biran et al., 2005; Cogan, 2008; Johnson et al., 2005; Otto et al., 2006; Williams et al., 2007). Impedance measurements at other frequencies within a particular MEA type displayed highly similar trends as the 1 kHz impedance measurements, which is the fundamental action potential frequency often used to probe tissue properties around an implanted microelectrode (Cogan, 2008; Karp et al., 2008; Ludwig et al., 2006). The figures therefore only present an averaged set of 1 kHz impedance values over all the channels of a particular MEA. Error bars represent the standard error weighted by the mean impedance. Day-1 represents 1× PBS pre-characterizations. Comparisons are limited due to significant sources of variability associated with comparing highly different MEAs and associated insertion protocols (refer to the Experimental procedures section and Table 4 for more details). The manufacturer suggested implant procedures were used to allow each array to perform at its optimal level, given that the surgical procedures were developed for this purpose and that each array’s geometry is unique. Implant locations were chosen to keep the larger floating arrays (Cyberkinetics Silicon, Cyberkinetics Iridium Oxide, and NeuroNexus Silicon MEAs) and smaller fixed arrays (Moxon Thin-Film Ceramic and TDT Microwire MEAs) implanted in highly similar regions. As a result, floating arrays were implanted in motor cortex and fixed arrays were implanted in visual cortex. Due to the large connector assemblies on the floating arrays, motor cortex was the best implant option to ensure normal mobility for the rat. Suggested bone screw locations for the fixed arrays, which required encircling the assemblies with screws, inhibited us from implanting the fixed arrays in the same area as the floating arrays.

There were highly disparate scarring responses from each MEA investigated. During the first few weeks following implantation, the microenvironment around an implanted MEA is constantly changing (Liu et al., 1999; Turner et al., 1999), accounting for the dramatic, highly variable measured impedances observed among MEA types. Large spikes in 1 kHz impedance were observed (∼1 MΩ on average) immediately post-operatively for the Cyberkinetics IrOx and Silicon arrays (Figs. 2A, C), followed by large drops in impedance the day after implantation (1 to 1.5 MΩ rise/fall on average). Impedances for the Cyberkinetics MEAs remained relatively constant until days 5 and 7, respectively, when impedances began to quickly increase. Peak impedances for the Cyberkinetics IrOx and Silicon arrays were 1.60±0.34 MΩ on day 0 (immediately post-operatively) and 1.40±0.18 MΩ on day 20, respectively. NeuroNexus Silicon (Fig. 2G) and TDT microwire (Fig. 2I) MEAs exhibited rather steady rises in impedance following implantation with peak impedances of 2.07±0.12 MΩ on day 15 and 0.27±0.01 MΩ on day 20, respectively. The Moxon Thin-Film Ceramic MEAs (Fig. 2E) displayed a decrease in impedance following implantation. This may reflect site damage upon insertion – most likely damaged ceramic insulation between sites – that increased the effective recording surface area. Impedance began rising on day 7, eventually peaking at 0.25±0.11 MΩ on day 20.

2.3. Analysis of neural recordings

Using OSort (Rutishauser et al., 2006), neural spike data was sorted for days 0, 10, 20, and 30 (when possible) for each MEA. Signal-to-noise ratios were calculated as previously described in (Suner et al., 2005).

\[
\text{SNR} = \frac{V_{pp,avg}}{2 \times \sigma_{noise}}
\]

In short, the SNR was calculated as the peak-to-peak voltage of the mean spike waveform \(V_{pp,avg}\) divided by 2× the standard deviation of the mean noise level \(\sigma_{noise}\).

Despite differing implant locations, and thus potentially differing levels of background neural activity, and differing scarring responses, which relates to the level of thermal noise in the observed signal (Ludwig et al., 2006), no significant SNR differences were observed among the MEA types for the duration of the study. Additionally, SNR trends did not seem to relate to site impedance trends over the 31-day period, and by inference, the extent of tissue encapsulation and neuronal density loss (Biran et al., 2005; Johnson et al., 2005; Otto et al., 2006; Williams et al., 2007). Fig. 3B shows relatively stable SNRs over time despite highly variable numbers of distinguishable single units and electrodes recording single units (Fig. 3A) throughout the same period.

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Table 3 – Summary of pre-characterization.

<table>
<thead>
<tr>
<th>MEA</th>
<th>Sites (MEAs) surveyed</th>
<th>Mean Qcap (mC/cm²)a</th>
<th>Mean (Z_{1kHz}) (kΩ)b-c</th>
<th>Func. sites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyberkinetics IrOx</td>
<td>64 (4)</td>
<td>10.4±0.84</td>
<td>74.1±12.4b</td>
<td>93.8</td>
</tr>
<tr>
<td>Cyberkinetics Silicon</td>
<td>96 (3)</td>
<td>6.10±0.12</td>
<td>9.75±1.63c</td>
<td>90.6</td>
</tr>
<tr>
<td>Moxon Thin-Film Ceramic</td>
<td>12 (3)</td>
<td>1.40±0.16</td>
<td>194±11.2c</td>
<td>100</td>
</tr>
<tr>
<td>NeuroNexus Silicon</td>
<td>64 (4)</td>
<td>0.80±0.02</td>
<td>184±36.8b</td>
<td>95.3</td>
</tr>
<tr>
<td>TDT Microwire</td>
<td>48 (3)</td>
<td>5.10±0.40</td>
<td>270±7.48b</td>
<td>100</td>
</tr>
</tbody>
</table>

a Charge capacity and impedance values are presented as (mean±std).
b Mean 1 kHz impedance in kΩ.
c Mean 1 kHz impedance in GΩ/cm².

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*Table 3 – Summary of pre-characterization.*
Fig. 4 presents randomly selected single units (data used for the SNR analyses) from each MEA type immediately postoperatively, and on days 10, 20, and 30 thereafter. Colors in the figure serve to distinguish among single units. All presented time axes are 2.5 ms in length. Spike amplitudes range from 60 to approximately 400 μV. Here, the ability to consistently record action potentials from multiple electrodes over time is the appropriate measure of electrode success or failure. As expected, MEAs with smaller baseline impedance values (indicating larger effective recording site areas), such as the Cyberkinetics IrOx and TDT Microwire MEAs, typically recorded more units with active sites than other MEAs. This is attributable to less recorded noise and less signal loss via 'shunt pathways' (Ludwig et al., 2006; Robinson, 1968). Those with larger baseline impedance values (indicating smaller effective recording site areas), such as the Cyberkinetics Silicon, NeuroNexus Silicon and Moxon Thin-Film Ceramic MEAs, typically recorded fewer units. Note that quantitative comparisons among MEAs implanted in highly different brain regions are not practical due to anatomical differences. These differences include neuronal density and cell types, glial density and cell types, and the number of active neurons at a given point in time. Limited comparisons can only be made between floating and fixed arrays (Table 2), which are implanted in highly similar regions (Table 4).

2.4. Immunohistochemical analysis

An immunohistochemical (IHC) analysis was performed for a rat implanted with each type of MEA. Brain slices obtained within 200 and 600 μm from the cortex surface (Figs. 5A, 6A, 7A, 8A, and 9A) were stained against general neurons (anti-
Table 4 – Summary of implanted rats.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Electrode/rat ID</th>
<th>Implant location</th>
<th>Implant order</th>
<th>Mass (g)</th>
<th>Sex</th>
<th>Days implanted</th>
<th>Reason for termination or death prior to 31 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyberkinetics, Inc.</td>
<td>CyberIrOx1290_2.a</td>
<td>M1, M2</td>
<td>15</td>
<td>310</td>
<td>M</td>
<td>26</td>
<td>Broken ref/gnd connection</td>
</tr>
<tr>
<td></td>
<td>CyberIrOx1290_2.b</td>
<td></td>
<td>2</td>
<td>280</td>
<td>F</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CyberIrOx1290_2.c</td>
<td></td>
<td>9</td>
<td>337</td>
<td>M</td>
<td>21</td>
<td>Lost headcap</td>
</tr>
<tr>
<td></td>
<td>CyberIrOx1290_2.d</td>
<td></td>
<td>10</td>
<td>350</td>
<td>M</td>
<td>31</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>CyberSil4530_.0004</td>
<td>M1, M2, Cg1</td>
<td>18</td>
<td>285</td>
<td>F</td>
<td>2</td>
<td>Complications from surgery</td>
</tr>
<tr>
<td></td>
<td>CyberSil4530_.0005</td>
<td></td>
<td>5</td>
<td>320</td>
<td>M</td>
<td>21</td>
<td>Damaged connector</td>
</tr>
<tr>
<td></td>
<td>CyberSil4530_.0006</td>
<td></td>
<td>11</td>
<td>360</td>
<td>M</td>
<td>21</td>
<td>Damaged connector</td>
</tr>
<tr>
<td>Moxon Thin-Film</td>
<td>Drexel2</td>
<td>V2MM</td>
<td>4</td>
<td>300</td>
<td>F</td>
<td>31</td>
<td>Broken ceramic shank</td>
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<tr>
<td>Ceramic</td>
<td>Drexel4.a</td>
<td></td>
<td>1</td>
<td>280</td>
<td>F</td>
<td>26</td>
<td>Lost headcap due to infection</td>
</tr>
<tr>
<td></td>
<td>Drexel5</td>
<td></td>
<td>6</td>
<td>326</td>
<td>M</td>
<td>16</td>
<td>Lost headcap</td>
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<tr>
<td></td>
<td>Drexel6</td>
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<td>8</td>
<td>312</td>
<td>M</td>
<td>31</td>
<td>n/a</td>
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<tr>
<td></td>
<td>Drexel7</td>
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<td>12</td>
<td>380</td>
<td>M</td>
<td>31</td>
<td>n/a</td>
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<tr>
<td>NeuroNexus, Inc.</td>
<td>NNexus1644</td>
<td>M1, M2</td>
<td>16</td>
<td>304</td>
<td>M</td>
<td>31</td>
<td>n/a</td>
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<tr>
<td></td>
<td>NNexus3602</td>
<td></td>
<td>7</td>
<td>311</td>
<td>M</td>
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<td>Lost headcap</td>
</tr>
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<td></td>
<td>NNexus4002</td>
<td></td>
<td>13</td>
<td>385</td>
<td>M</td>
<td>31</td>
<td>n/a</td>
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<td>14</td>
<td>286</td>
<td>M</td>
<td>31</td>
<td>n/a</td>
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<tr>
<td>Tucker-Davis</td>
<td>TDT3235</td>
<td>V2MM, V2ML</td>
<td>3</td>
<td>300</td>
<td>F</td>
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</tr>
<tr>
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<td></td>
<td>17</td>
<td>291</td>
<td>F</td>
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<td>330</td>
<td>F</td>
<td>31</td>
<td>n/a</td>
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</tbody>
</table>

* These animals were not included in the final data set due to known complications with a surgical procedure or data collection equipment.

Beta III Tubulin (anti-GFAP) and reactive astrocytes (anti-GFAP). Slices obtained within 600 and 1200 μm from the cortex surface (Figs. 5B, 6B, 7B, 8B, and 9B) were stained against rat monocyes/macrophages (anti-CD68) and reactive astrocytes (anti-GFAP). All images are presented with the tops of the images most rostral and the bottom of the images most caudal.

A fibrous encapsulation was found around all of the explanted MEA interfaces. Each electrode elicited a unique recruitment of immune effector cells. Due to the low sample number for the immunohistochemical analysis (n=1/MEA type) and differing implant locations for floating and fixed arrays, no quantitative comparisons are made between groups. Rather, the images are presented to emphasize the highly variable immune responses encountered from current MEA technology.

Representative histological images of brains implanted with Cyberkinetics IrOx and Silicon MEAs are shown in Figs. 5 and 6, respectively. A large fibrous mass of scar tissue is seen in Fig. 5A and Fig. 6A that is not seen in images from other MEA types. The Cyberkinetics IrOx MEAs display higher levels of astrocyte and monocyte/macrophage recruitment than the Cyberkinetics Silicon MEA images. However, the large amount of scar tissue seen in Fig. 6A indicates that there was a more adverse reaction to the Cyberkinetics Silicon MEA selected for the histological analyses.

Both Cyberkinetics MEAs are wired to 36-pin male Omnetics Nano connectors, which are rather large for use in rats. Because of this, we were forced to implant much more rostral than we had anticipated. In at least one instance for each Cyberkinetics MEA type, one or more electrode shanks pierced a major blood vessel, potentially recruiting macrophages from systemic blood flow and eliciting a stronger overall immune response to the implants (Polikov et al., 2005; Schmidt et al., 1993). This likely occurred in the Cyberkinetics MEA-implanted rats selected for histological analyses.

The Moxon Thin-Film Ceramic MEA histology images (Fig. 7) display the most localized immune response, with a clear, well-defined astrocytic encapsulation surrounding the explanted MEA interface (Fig. 7B). This figure clearly shows a strong astrocytic response with little or no visible monocyes/macrophages present. The slice from Fig. 7B was obtained from ~1000 μm below the cortex surface in the vicinity of the Cr/Pt recording sites. Fig. 7A, obtained within 600 μm of the cortex surface, shows much less astrocytic recruitment and a large cavity. The probe was loose within the cavity upon removal (this was also observed in other animals implanted with this probe that were not selected for histology). The cavity may have been caused by chronic micromotion of the brain relative to the probe (Kim et al., 2004b), which is ‘fixed’ to the skull and bone screws using dental cement.

The NeuroNexus MEA histology images display the most visible monocyte/macrophage recruitment along with a strong astrocytic response (Fig. 8B). The large hole left behind by the MEA (~125 μm wide and ~200 μm long) along with the non-uniform neuron staining (Fig. 8A) is evidence of damage from probe explantation, likely due to strong scar tissue adhesion to the implant (Liu et al., 1999). Interestingly, although both NeuroNexus and Cyberkinetics MEAs were implanted in motor cortex, the NeuroNexus MEAs displayed highly dissimilar impedance trends in comparison to those of the Cyberkinetics MEAs (no spikes in impedance immediately after implantation). These differences are addressed in the Discussion section.

The TDT Microwire MEA histology images show moderate astrocyte and monocyte/macrophage recruitment. Fig. 9A shows evidence of tearing near the cortex. The torn appearance of the insertion site is not visible in other MEA images, and may be a result of the cortical compression or the ‘dimpling’ effect, seen during the slow insertion of grid arrays.
response to each implant is highly apparent in the histology images from Figs. 5–9 and the 1 kHz impedance trends in Fig. 10. While Figs. 5–10 do not necessarily represent typical responses to the implanted MEAs, the observed variability among the responses is typical.

At the measured time points (days 0, 10, 20, and 30), SNRs were relatively stable for all MEA types evaluated. Two possible explanations are discussed. In vitro, thermal noise is often the main component of the observed noise since little or no background neuronal activity is present. If it is also the dominant source in vivo, the SNR trends are expected to follow the impedance trends, since thermal noise is directly proportional to the square root of electrode impedance (Ludwig et al., 2006). Since SNR trends did not follow impedance trends in this study, noise in the recordings likely came predominantly from background electrical activity, not electrode thermal noise (Cogan, 2008) (Fig. 3B shows that higher thermal noise levels expected from higher impedance electrodes do not lower the SNRs when compared to lower impedance electrodes, supporting the notion that it is not the dominant noise source within this study).

It is also possible that long, unshielded leads between the high impedance electrodes and the high input impedances of the recording amplifiers can pick up additional background activity (remote neural activity or random noise) when placed sufficiently close to highly active regions of the cortex. These long, unshielded leads can decrease SNR by receiving variable levels of background noise independently of the recording sites, depending on the implant location and path of the MEA lead wires (Bai and Wise, 2001; Hetke et al., 1994). Therefore, unless thermal noise or noise from electronics becomes the dominant noise source, the SNR of a particular single-unit recording is expected to decrease if the lead wires continue to transmit background neuronal chatter independently of the recording sites while the signals sensed by the recording sites decrease in amplitude due to the effects of local neurodegeneration, neural migration away from the recording site, and electrical isolation from the highly resistive encapsulation (Purcell et al., 2009).

3.1. Floating arrays

Among the floating arrays compared in this study (Cyberkinetics IrOx, Cyberkinetics Silicon, and NeuroNexus Silicon MEAs), possible comparisons are hindered by overall MEA composition, size, insertion method, whether the MEAs have one or multiple shanks, and whether they record from one or multiple planes (see first 3 columns of Table 5). In the first few days following implantation, both Cyberkinetics MEAs showed similar trends in measured 1 kHz impedances over the duration of implantation (Fig. 10) that were markedly different from the trends observed among other MEA types. The most noteworthy portions of the Cyberkinetics MEA impedance trends are the large spikes in impedance seen immediately after implantation (on day 0) followed by nearly equivalent drops in impedance seen on day 1. Impedance rises of 70 to 100 kΩ have been observed by other researchers immediately after insertion when using NeuroNexus Silicon MEAs, but these were not followed by large drops in impedance as was seen with both Cyberkinetics MEAs. In

Sample sizes for each MEA type in this investigation are small, but sufficient for providing insights into MEA-specific characteristics and areas requiring further investigation. Possible statistical analyses are hindered by several potential sources of variability, however, impeding many comparisons between MEAs. Table 5 lists many of the major sources of variability seen within and between MEA types in this study. It is organized to highlight conditions that were kept consistent between MEAs and differences that may contribute to the overall observed variability in responses within and between MEAs. Much of the variability, as with any study, is attributable to procedure and the highly variable and somewhat unpredictable in vivo environment in which the MEAs are evaluated (Liu et al., 1999). The variability in the scarring

Fig. 3 – General analysis of MEA recording characteristics. (A) Percent of active recording sites over the 31-day implantation period. (B) Mean SNR over the 31-day implantation period. Error bars indicate standard errors within a particular MEA. No data was obtained on day 30 for the Cyberkinetics Silicon MEAs, because the connectors were too damaged.
general, it is thought that post-operative impedance changes seen within the first few days are attributable to adsorbed soluble factors and a differing ionic environment from pre-characterization conditions (Ludwig et al., 2006). The post-operative impedance change magnitudes observed from both Cyberkinetics MEA types were much larger than those observed from other MEAs (1 to 1.5 M\(\Omega\) rise/fall on average), indicating that geometry or insertion method/conditions may be important factors that contributed to the observed response. A more gradual rise in impedance is observed among the NeuroNexus Silicon MEAs, which show no adverse responses immediately after insertion and follow similar trends to the Cyberkinetics MEAs after day 1. Turner et al. (1999) showed that the glial scar stabilizes around a planar silicon shank approximately two weeks after implantation, transitioning into the chronic phase thereafter (Turner et al., 1999). The impedance trend for the NeuroNexus Silicon MEA (but not the Cyberkinetics Silicon MEA) agrees with these findings.

The large spikes in impedance seen from both Cyberkinetics MEAs do not seem to affect the MEAs recording quality, as the mean SNRs immediately after insertion were not significantly different from other MEAs. Along with NeuroNexus, however, the Cyberkinetics MEAs had the lowest percentage of sites recording single units immediately post-operatively (day 0). This may be due to tissue displacement, swelling, or other effects of the early phases of the foreign body response, which are thought to affect recording stability in the first several days following implantation (Liu et al., 1999; Ludwig et al., 2006; Turner et al., 1999).

3.1.1. Cyberkinetics IrOx MEA

The Cyberkinetics IrOx MEA exhibited the highest overall mean charge capacity, likely due to its large activated Iridium Oxide recording tips (~760 \(\mu\)m\(^2\) uncoated). The thin Iridium Oxide coating can increase the charge capacity by one order of magnitude by increasing the effective surface area of the electrode (Robblee et al., 1983; Weiland and Anderson, 2000).

Fig. 4 – Sorted single units recorded using each MEA type immediately post-operatively, as well as days 10, 20, and 30 thereafter. Each window spans 2.5 ms. Action potential amplitudes ranged from approximately 60 to 400 \(\mu\)V.
This means that it is capable of delivering more charge to neural tissue in a reversible manner than the other MEA types investigated. Also, due to the low impedance of the Iridium Oxide electrodes, less signal loss is expected through shunt pathways (Ludwig et al., 2006; Robinson, 1968). One tradeoff, however, is the inconsistent charge capacity. Another is that stimulation is restricted to one plane due to the inherent fixed insertion depth of the MEA shanks.

The large post-implantation spikes in impedance observed with the Cyberkinetics MEAs (but no other MEAs) suggest that the fast insertion method, substrate composition/insulation, or severe hemorrhaging resulting from insertion of the relatively large device triggered the phenomenon (see first 3 columns of Table 5). Since the TDT Microwire MEA, which also has its recording sites in a planar grid configuration, did not elicit a similar response, cortical compression is likely not the cause. It is also unlikely that the substrate or parylene C insulation triggered the phenomenon, because their chronic presence would only further exacerbate the observed response (which would be apparent in the measured impedance throughout the study period). As mentioned before, the large size of the Cyberkinetics MEA assembly relative to a rat’s skull made it difficult to find an appropriate implant location. Despite considerable efforts to avoid implanting an MEA in highly vascularized region, a major blood vessel was often pierced during the fast insertion of the array, resulting in

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**Fig. 5** – Histological evaluation of a rat brain implanted with a 16-channel Cyberkinetics IrOx MEA (Rat ID: CyberIrOx1290_2_d). (A) Brain slice labeled with anti-GFAP (red) and anti-beta-III tubulin (green). Note the fibrous scar tissue in the top half of the image. (B) Brain slice labeled with anti-GFAP (red) and anti-CD68 (light blue).

**Fig. 6** – Histological evaluation of a rat brain implanted with a 32-channel Cyberkinetics Silicon MEA (Rat ID: CyberSil4530_0005). (A) Brain slice labeled with anti-GFAP (red) and anti-beta-III tubulin (green). Note the fibrous scar tissue in most of the image. (B) Brain slice labeled with anti-GFAP (red) and anti-CD68 (light blue). Arrows point to discernible holes where the MEA shanks were implanted.
hemorrhaging. An abundance of organic material introduced from this hemorrhaging likely adsorbed to the surface of the recording sites immediately after insertion, increasing the observed impedance (Ludwig et al., 2006; Yousif et al., 2008).

We do not have an explanation for the sudden impedance drop seen the day after implantation.

3.1.2. Cyberkinetics Silicon MEA

The Cyberkinetics Silicon MEA exhibited the second highest overall mean charge capacity. In all cases, there was severe bleeding after insertion, indicating moderate to several vascular trauma (Ludwig et al., 2006; Polikov et al., 2005; Schmidt et al., 1993). The large, fibrous encapsulation seen in

Fig. 6A further supports this observation. Post-implantation impedance trends, highly similar to those of the Cyberkinetics IrOx MEA, were also observed for this MEA type. As mentioned previously, however, these spikes in impedance did not adversely affect the MEAs recording quality, but the number of sites from which discriminable single-unit activity could be recorded. Like the Cyberkinetics IrOx MEA, the Cyberkinetics Silicon MEA is limited to recording and/or stimulating from a single plane due to its planar grid configuration.

Fig. 7 – Histological evaluation of a rat brain implanted with a 4-channel Moxon Thin-Film Ceramic MEA (Rat ID: Drexel7). (A) Brain slice labeled with anti-GFAP (red) and anti-beta-III tubulin (green). (B) Brain slice labeled with anti-GFAP (red) and anti-CD68 (light blue). Note the highly organized network of reactive astrocytes and the well-defined outer perimeter of the encapsulation.

Fig. 8 – Histological evaluation of a rat brain implanted with a 16-channel NeuroNexus Silicon MEA (Rat ID: NNexus1644). (A) Brain slice labeled with anti-GFAP (red) and anti-beta-III tubulin (green). (B) Brain slice labeled with anti-GFAP (red) and anti-CD68 (light blue). Note the well-defined halo of macrophages/monocytes surrounding the large cavity left behind by the MEA. These images show more extensive damage from explantation than similar images from other MEA types. The arrow indicates a hole left by an individual shank of the MEA.
3.1.3. NeuroNexus Silicon MEA
The NeuroNexus Silicon MEAs exhibited the lowest overall mean charge capacity. However, they also had the lowest site-to-site variability, indicating excellent quality control during the manufacturing process. This MEA is useful if consistent site-to-site properties are a concern and if recordings must be obtained from multiple depths of the cortex. Also, since the recording sites are made from Iridium, investigators can form oxide coatings in their labs prior to implantation so that the recording sites have consistent, high charge injection capabilities when implanted. One method of activating Iridium electrodes to form a controlled oxide coating is outlined in Liu et al. (1999).

3.2. Fixed arrays
Among fixed arrays compared in this study (Moxon Thin-Film Ceramic and TDT Microwire MEAs), possible comparisons are hindered by overall composition, dimensions, whether the MEAs have one or multiple shanks, and whether they record from one or multiple planes (see last two columns of Table 5). The scarring responses of brains implanted with the fixed MEAs are highly different. While the observed responses may not be typical, the images (Figs. 7, 9) serve as more examples of the unpredictable scarring responses plaguing current MEA technology.

3.2.1. Moxon Thin-Film Ceramic MEA
The Moxon Thin-Film Ceramic MEA had a low overall charge capacity and high variability in site-to-site impedance. This means that it may not be a good MEA for applications requiring stimulation, since the MEA sites can easily become damaged when large currents are needed and because site properties are inconsistent. Since this MEA is not commercially available and made using an elaborate manufacturing process (Moxon et al., 2004a; Moxon et al., 2004b), small-scale production likely contributes to the significant variability in site-to-site properties. Insertion of this MEA was rather straightforward. However, due to their small size and the brittle nature of ceramics, several MEAs were broken prior to a successful implantation. Despite the high degree of variability within this MEA type, the Moxon Thin-Film Ceramic MEA was not outperformed by the other MEA types investigated. This MEA is useful for recording from multiple planes.

3.2.2. TDT Microwire MEA
The TDT Microwire MEAs displayed the lowest overall impedances due to their large recording site areas ($\sim 2300 \mu m^2$) and a mean charge capacity similar to that of the Cyberkinetics Silicon MEAs ($5.1 \pm 0.4 \mu C/cm^2$ for TDT microwire MEAs and $6.10 \pm 0.12 \mu C/cm^2$ for Cyberkinetics Silicon MEAs). Their large recording site areas permit them to record from larger groups of neurons, yielding more single units per channel than other electrodes. Insertion was simple, but the microwires deviated from their intended trajectory (Fig. 9B) (recording sites should be evenly spaced at their final resting position) and tore through the cortex (Fig. 9A). During the initial stages of the slow insertion, there was a noticeable compression of the cortex before a sudden relaxation of the neural tissue around the individual microwires (Campbell et al., 1991; Jones et al., 1992). The torn appearance of the tissue near the cortical surface is likely a result of the sudden entry of the relatively large microwires in the array into the neural tissue after it had been compressed several hundred micrometers (Campbell et al., 1991; Edell et al., 1992; Jones et al., 1992). This array is suitable for applications in which large populations of neurons in a single plane are of interest (although the MEA can be configured to record from multiple depths). The arrays are also useful for stimulating large groups of neurons. Precise implant locations may be difficult to target, however, if deviations from the intended trajectory are typical.

![Histological evaluation of a rat brain implanted with a 16-channel TDT Microwire MEA (Rat ID: TDT3496).](image)
3.3. Limitations and future directions

All MEAs evoke highly variable scarring responses, especially in the first 6 to 8 weeks after implantation (Polikov et al., 2005; Yousif et al., 2008). This makes recording and stimulation unpredictable (Yousif et al., 2008). Most of the current designs are based on mechanical considerations that minimize tissue trauma (e.g., electrode sharpness, shape, size, and insertion speed), material selections that minimize the observed foreign body response (e.g., exposed surface features and biocompatibility), and design features that are necessary to record from or stimulate many small populations of neurons (e.g., recording site area, spacing, configuration, and charge injection capabilities). Despite countless design efforts, a consistent, tolerable response to an implant has not been achieved (Polikov et al., 2005).

Previous studies demonstrated that the scarring response is triggered or exacerbated by an inherent foreign body response to the implanted material penetrating the blood-brain barrier (Biran et al., 2005; Turner et al., 1999), micro-motion of the implant relative to the brain (Kim et al., 2004b), and/or innate responses to trauma from insertion (Bjornsson et al., 2006; Edell et al., 1992). The relative contributions of these effects have not been substantially quantified, however, partly due to the high degree of variability in the foreign body response and partly due to the difficulties associated with assaying the various components of the encapsulation (Cogan, 2008; Yousif et al., 2008). This study provides several examples of the unpredictability of the foreign body response to an implanted MEA and highlights the need for a better understanding of the underlying mechanisms that trigger this response. Since multiple design efforts have not led to an MEA with a predictable response in long-term applications, it would be useful to define general relationships between MEAs mechanical properties, electrical properties, and the observed foreign body response (Polikov et al., 2005). Knowledge of such relationships can help array designers to develop a more standardized microelectrode design with minimal adverse effects after implantation.

<table>
<thead>
<tr>
<th>Source</th>
<th>Floating arrays</th>
<th>Fixed arrays</th>
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<tr>
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<tr>
<td>Experience</td>
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<tr>
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<td>MEA type (fixed vs floating)</td>
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</tr>
<tr>
<td>MEA geometry (single vs multiple shanks)</td>
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<td>Insertion method (fast vs slow)</td>
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<td>MEA substrate insulation</td>
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<tr>
<td>MEA geometry (dimensions)</td>
<td>See Table 1</td>
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</table>

MEAs are grouped based on whether they are classified as floating (where electrodes are not fixed to the skull to allow for movement with the brain) or fixed (where electrodes are fixed to the skull via dental cement and several bone screws). An ‘x’ across a row indicates that the condition remained consistent for each MEA type. An italicized row within the floating or fixed MEA group indicates a potentially major source of the observed variability between MEAs in the floating or fixed MEA group, respectively.

Fig. 10 – Summary of mean 1 kHz impedances for each MEA over the duration of implantation.
While electrochemical impedance spectroscopy (EIS) is quite insightful, its interpretation is not well developed as a result of a non-standardized microelectrode design, and furthermore, as a result of the incomplete understanding of the underlying mechanisms leading to implant failure (Cogan, 2008; Polikov et al., 2005; Polikov et al., 2006). Many researchers are investigating the relative contributions of components of the foreign body response to observed changes in complex impedance spectra. Due to uncontrolled variability in animal models, much of this work is performed in vitro where the environment is more controlled (Polikov et al., 2006). One group has shown in vitro that different proteins involved in the foreign body response and novel targets for intervention. Selection of an MEA for use with a chronically implantable medical device (such as an epilepsy prosthesis) today will depend heavily on implant location, duration, and the desired recording and/or stimulation characteristics (e.g., selection of Moxon Thin-Film Ceramic or NeuroNexus Silicon MEAs for highly selective recording/stimulation across multiple layers of the cortex, or selection of the Cyberkinetics IrOx, Cyberkinetics Silicon, or TDT Microwire MEAs for recording from or stimulating neurons in a single plane). It is hoped that this study will facilitate funding for further research in these areas so that more reliable, standardized MEAs can be developed.

5. Experimental procedures

5.1. Design

A simple experimental paradigm was chosen due to a limited experimental budget and the difficult nature of comparing highly dissimilar microelectrode configurations in vivo. Up to 4 MEAs of a particular configuration were evaluated to help quantify manufacturing variability, and furthermore to reduce procedural and biological variability. Prior to implantation, each MEA was characterized in 1× PBS, including analyses of site impedance and charge capacity. After thorough training, a lead surgeon (one of two) implanted MEAs into a total of 19 rats. Implantation procedures were performed in a random order to help minimize potential biases. Implanted MEAs were then monitored over a 31-day period, specifically after implantation and on days 1, 2, 3, 5, 7, 10, 15, 20, 25, and 30 thereafter. A time-span of 31 days is sufficient to maximally capture the acute and early phases of the chronic immune response to an implanted MEA (Yousif et al., 2008). Site impedances and recordings were collected on each data collection day. After collecting data on day 31, a representative rat of each MEA type was selected for a histological analysis in order to observe any elicited immune response. Only 1 rat implanted with each MEA type was selected to help keep study costs within affordable limits.

5.2. Surgical training

The lead surgeon received training by NeuroNexus Technologies and Cyberkinetics Neurotechnology Systems prior to performing any NeuroNexus Silicon, Cyberkinetics IrOx, or Cyberkinetics Silicon MEA implantations. NeuroNexus MEA insertion training took place in January 2007 at Michigan University (Ann Arbor, MI) and Cyberkinetics MEA insertion training took place in July 2006 at Brown University (Providence, RI). All Moxon Thin-Film Ceramic MEA implantations followed a manufacturer recommended surgical procedure adapted from Moxon et al. (2004b) and all TDT Microwire MEA implantations followed a manufacturer recommended surgical procedure adapted from Kralik et al. (2001).

5.3. Surgical procedures

A total of 19 Long Evans rats were implanted with unique MEAs using manufacturer recommended insertion protocols (Kipke et al., 2003; Kralik et al., 2001; Moxon et al., 2004b; Rousche and Normann, 1998). Implant location depended on MEA type,
configuration, and available space, but remained consistent for each type of array. Ideally, the variety of MEAs should be implanted in the same brain region of the same animal using the same insertion method in order to eliminate effects from differing populations of neurons and glial cells as well as from insertion method. Unfortunately, size limitations and recommended surgical procedures (which were developed to maximize the performance of the MEAs) prohibit this option, thereby limiting the conclusions that can be inferred from the data. To allow for direct comparisons between MEAs with the aforementioned limitations, Moxon Thin-Film Ceramic and TDT Microwire MEAs were implanted in left visual cortex, mediomedial area (V2MM). The larger size of the TDT MEAs required some sites to be implanted in the mediolateral area of the left visual cortex (V2ML). In general, fixed arrays such as the Moxon Thin-Film Ceramic and TDT Microwire MEAs were implanted in the visual cortex to allow room for several bone screws (used as anchors to fix the arrays in place). The 32-channel Cyberkinetics Silicon-based MEAs were positioned such that electrode sites were implanted in right and left M1 and M2. A summary of the animals included in this study is shown in Table 4. Only 16 of the animals are represented in the results. Animals were excluded due to known complications with the surgical procedures, data collection equipment, or infections. Three of the 19 animals were not included in the final data analysis due to a broken ref/gnd connection (CyberIrOx1290_2_a), a broken shank (Drexel2), and a lost headcap due to infection (Drexel4). Early termination (6 out of 19 animals) was due to lost headcaps (CyberIrOx1290_2_c, Drexel5, and NNexus3602), an unknown complication from surgery (CyberSil4530_0004), and an inability to interface with badly damaged connectors (CyberSil4530_0005 and CyberSil4530_0006).

All surgical and animal handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC) prior to performing the study and adhered to the guidelines for the care and use of laboratory animals. Anesthesia was induced via 5% Isoflurane in 2 L/min O₂ or an anesthetic cocktail consisting of 75–95 mg/kg Ketamine and 5 mg/kg Xylazine administered intraperitoneally. A surgical plane was maintained via 0.5 to 3% Isoflurane in 2 L/min O₂ (Swindle et al., 2002). Post induction, the surgical site was shaved and cleaned with alternating scrubs of Dial Surgical Scrub and Betadine. Using a standard stereotactic frame, a midline incision was made and the skull was cleaned to expose lambda, bregma, and the proposed craniotomy site. Bone screw locations and the proposed craniotomy site were marked prior to drilling with a sterile ruler and cautzerizer. Up to 4 support and 1 REF/GND stainless steel bone screws were used per animal to affix the array to the skull and to use as a return path for FRA or a ground during recordings. Craniotomies were drilled after inserting any support screws to help minimize cortex exposure time. All probes were sterilized in a 70% ethanol in DH₂O solution prior to insertion. NeuroNexus and Cyberkinetics arrays were fixed to the support screws using dental cement prior to inserting the electrodes. After inserting each array, the remaining exposed cortex or dura mater was covered along with exposed electrode shanks using Kwik-Sil silicone elastomer. Dental cement was used to cover the remaining exposed, pre-cleaned skull surface to help ensure the optimal lifetime of the headcap (Gardiner and Toth, 1999). The Cyberkinetics arrays were inserted according to manufacturer recommendations using their pneumatic inserter in order to achieve an insertion depth of 1 mm at high velocity (Rousche and Normann, 1992). Recordings and impedance data were collected post-operatively to ensure probe viability. Additional details of the insertion protocols for each MEA are found in Kipke et al. (2003), Knalik et al. (2001), Moxon et al. (2004b), and Rousche and Normann (1998).

5.4. Data collection

All probes were characterized prior to implantation in a 1× PBS (pH=7.4) solution via electrochemical impedance spectroscopy (EIS). Specifically, frequency response analyses and cyclic voltammetry (FRA and CV, respectively) were performed on each electrode site to identify starting impedances, charge capacities, and any damaged or broken sites. An Autolab PGSTAT12 potentiostat was used for all CV analyses using 3 cycles with a sweep range of −0.6 to 0.8 V and a sweep rate of 1 V/s. A sweep range of −0.6 to 0.8 V was chosen due its wide acceptance as the water electrolysis window (vs Ag/AgCl) for Platinum and Iridium Oxide. It is broad enough to capture reversible redox reactions without damaging the MEAs (Cogan et al., 2005; Richardson-Burns et al., 2007). A sweep rate of 1 V/s was chosen based on previous studies (Johnson et al., 2005; Otto et al., 2006) and yielded sufficient data to determine the charge capacity of each recording site. A Pt counter electrode and Calomel reference electrode were used to complete a 3-electrode setup during CV analyses. A single custom 32-channel multiplexing headstage and accompanying software, designed to handle all MEA types, was used to obtain all impedance data and recordings throughout the study. Impedances, referenced to a stainless steel bone screw in a two-electrode setup (Williams et al., 2007), were obtained from 0.1 to 10 kHz using fixed 100 mVp transport sinusoidal waveforms. The reference wire for each probe was used as a return path for the input sinusoid where reactance and resistance data were extracted (2005; Williams et al., 2007).

Barring complications, impedance data and recordings were collected immediately post-operatively and on days 1, 2, 3, 5, 7, 10, 15, 20, 25, and 30 after implantation to monitor the tissue responses to the MEAs (Williams et al., 2007). Typically, animals were lightly anesthetized via 3–5% Isoflurane in 2 L/min O₂ prior to data collection to minimize artifacts from movement and to help gather more reliable impedance data. After recovering, recordings were taken for 1 to 3 min per electrode site using a 25 kHz sampling rate.

5.5. Immunohistochemical analysis

Immediately following data collection on day 31, an animal implanted with each type of MEA was perfused using a 4% (w/v) paraformaldehyde in 1× PBS solution following previously published methods (Shain et al., 2003; Turner et al., 1999). Post perfusion, the brain and probe were carefully extracted and post-fixed in 4% paraformaldehyde at 4°C for an additional 24 h, followed by 3 rinses in 1× PBS at 4°C over the course of 24 h. Fixed brains were then transferred to Hank’s Buffered HEPES Solution (HBHS) with
sodium azide (90 μg/mL) and stored at 4 °C until they were ready for processing (Shain et al., 2003). A Leica VT1000 S vibratome was used to obtain 100 μm sections perpendicular to the electrode insertion sites (Williams et al., 2007). Each slice was collected and transferred to a 24-well plate containing 1 mL HBHS per well.

Specimen preparation closely followed previous staining procedures developed in Shain et al. (2003) and Turner et al. (1999). Reactive astrocytes were stained using a rabbit anti-glial fibrillary acidic protein (GFAP) polyclonal primary antibody in a 1:500 stock dilution with HBHS and an Alexa Fluor 635 nm goat anti-rabbit IgG (H+L) secondary antibody in a 1:200 stock dilution with HBHS. Reactive microglia were stained using a mouse anti-ramonocytes/macrophages (CD68) monoclonal primary antibody in a 1:500 stock dilution with HBHS and an Alexa Fluor 488 nm goat anti-mouse IgG1 (γ1) secondary antibody in a 1:200 stock dilution with HBHS. A general neuron stain was performed using a mouse anti-beta III tubulin affinity purified polyclonal antibody in a 1:200 stock dilution with HBHS and a fluorescein conjugated affinity purified secondary antibody in a 1:100 stock dilution with HBHS. Stained specimens were imaged using 488 nm and 633 nm laser lines on an Olympus IX81 inverted laser confocal microscope.

5.6. Data analysis

All impedance, CV, and neural recording data sets were analyzed in Matlab R2007a under identical conditions. All CV and impedance data sets were averaged across all channels within a particular type of MEA. Basic statistics such as variance and standard error were calculated concurrently. Charge capacity was determined by calculating the average area under each CV waveform from −0.6 to 0.8 V and dividing it by the electrode site area (units of mC/cm²).

All presented neural recording data was sorted using a modified version of Ueli Rutishauser’s freely available spike-sorting package OSort. In brief, the software first band-pass filters the raw signal from 0.3 to 3 kHz. A running average of the power in the signal is then calculated. If the local power of the filtered signal is at any point greater than 3× the standard deviation of the average signal power, a 2.5 ms window is extracted from the filtered signal, upsampled to 100 kHz, and sorted. Additional details are found in Rutishauser et al. (2006).

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